Strongly bonded family members in common marmosets show synchronized fluctuations in oxytocin

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Abstract

Oxytocin is a key regulator of social bonding and is positively linked to affiliation and prosocial behavior in several mammal species. In chimpanzees, this link is dyad-specific as affiliative interactions only elicit high oxytocin release if they involve strongly bonded individuals. These studies involved isolated dyads and sampling events. Little is known about the role of oxytocin in affiliation and social bonding, and about potential long-term patterns of bonding-related and dyad-specific oxytocin effects within highly affiliative and cooperative social groups. Our aim was to investigate whether bonding-related oxytocin signatures linked to dyadic affiliation are present in family groups of cooperatively breeding marmoset monkeys (*Callithrix jacchus*) that show high levels of cohesion and cooperation. In 30 dyads from four family groups and one pair, we measured urinary baseline oxytocin over six weeks and analyzed the link to bond strength (mean dyadic affiliation). Strongly bonded dyads showed synchronized longitudinal fluctuations of oxytocin, indicating that dyad-specific oxytocin effects can also be traced in the group context and in an interdependent species. We discuss these results in light of the potential function of differentiated relationships between marmoset dyads other than the breeding pair, and the role of oxytocin as mediator for social bonding.

Key words: oxytocin synchrony, social bonding, group-living, affiliation, marmoset monkeys

Introduction

The neurohormone oxytocin (OT) plays an important role in the regulation of mammalian social bonds and bonding related behaviors [1-7]. Its functions presumably have been expanded from the more ancient mother-infant and sexual bonds to a wider range of relationships in highly social species, including primates [8, 9]. A broad body of evidence suggests a positive link between OT and affiliative or prosocial interactions, as for example in rats [10], meerkats [11], and humans [12]. In the latter, elevated peripheral OT levels correspond with increased trust, and intranasal OT administration facilitates social perception and trust [13-16].

In non-human primates, OT correlates have been mainly studied in callitrichid monkeys and chimpanzees. Increased urinary OT concentrations accompany socio-sexual and affiliative interactions in both species [17, 18] and food sharing events in chimpanzees [19]. The results in chimpanzees have shown that such effects can be dyad-specific, in that increased urinary OT levels were detected only after affiliative interactions between socially bonded individuals, but not between non-bonded individuals [18, 19]. Individuals in chimpanzee societies form strong and long-lasting social bonds that are thought to entail direct and indirect fitness benefits [20, 21], and OT responses related to specific group members have been suggested to facilitate non-cognitive bookkeeping of social relationships [18].

The bonding-dependent release of OT during affiliative interactions is also consistent with experimental results. Intranasal and intracerebral OT administration affects partner preference [22, 23] and paternal food transfer in marmosets [24]. The experimental increase in OT thus mimics the effect of affiliative interactions with bonded individuals. As a result, subjects treat the experimental partners like stronger bonded individuals. The effects of OT application in humans can also be interpreted this way [13-16].

Crockford et al. [18] showed that OT secretion following dyadic affiliation is higher in strongly bonded dyads compared to interactions in less bonded dyads. Consequently, an individual's OT profile should be particularly determined by its affiliative events with strongly bonded group members, whereas interactions with other group members should influence its OT profile less. Over time, this should lead to temporal correlations in the OT profiles of bonded dyads, i.e. synchronized peaks and troughs of baseline OT, reflecting how much the bonded individuals are engaging in affiliative behavior with each other. No such tendency toward synchronized changes over time should occur in non-bonded dyads, because affiliative interactions in such dyads is expected to have no or a much smaller effect on OT. The aim of our study was to investigate the degree of dyadic OT synchrony in family groups of cooperatively breeding marmoset monkeys, in which affiliative interactions are frequent among all group members and some individuals form strong dyadic relationships [25, 26]. Marmoset groups are typically composed of a dominant breeding pair, several adult helpers, and immatures [27, 28]. Relationships between breeding pairs involve intense partner-directed affiliative and sexual behavior [17, 25, 26, 29, 30], and are characterized as socially monogamous [30-37]. The relationships between other group members are mostly peaceful and cooperative [26], even though punctual events of severe aggressions occur, in particular related to competition for breeding positions [38].

Specifically, we investigated the link between urinary baseline OT and dyadic affiliation in family groups of common marmosets (*Callithrix jacchus*) in order to test whether dyad-dependent OT response patterns are traceable within the family environment in this species, and whether temporal changes in baseline OT are synchronized in dyads, depending on their bond strength. If so, OT synchronization was expected to be most pronounced in stronger bonded

marmoset dyads (estimated based on mean dyadic affiliation). This should especially apply to breeding pairs where specific monogamous bonds are accompanied by high levels of affiliation and socio-sexual behavior [25, 30], but it may also occur in non-breeding individuals, whose relationships with other group members have barely been studied so far.

Urinary OT levels provide a proxy for peripheral OT, and have the advantage that they can be repeatedly sampled noninvasively, and thus without interrupting the social dynamics within the groups through invasive handling. The relevance of peripheral OT measures for studying the dynamics of social relationships has been recently reviewed and discussed by Crockford et al. [39]. OT is synthesized in various peripheral organs as well as in the hypothalamus, from where it is released peripherally and centrally [40, 41]. Several studies suggest a direct link [1, 11, 42, 43] as well as coordination between both systems based on neuroanatomical and physiological evidence [44-46]. This link is probably also triggered indirectly, for example via steroid hormones [47-49] and bidirectional feedback mechanisms with peripheral organs and body states [41], but the topic remains debated [5, 50]. Whereas cerebrospinal fluid (CSF) and plasma OT levels are positively correlated in humans [51], no such link was found in goats [52], pig-tail macaques [53], and lactating rhesus monkeys [54], suggesting independent control mechanisms of central and peripheral OT secretion. However, whether the release of central and peripheral OT is coordinated, seems to also depend on the involved stimulating brain regions (e.g. in the paraventricular nucleus) [55]. This indicates high flexibility and context-dependent activation of central and peripheral OT pathways. Several clearance studies support a correlation between systemic and urinary OT changes [56-58]. In various species, including humans and non-human primates, both plasma and urinary OT levels have been found to correspond with social environmental stimuli [5-7, 17-19, 56, 58-60] or

physiological and psychological states [59, 61-63]. In contrast to short-term OT fluctuations in plasma and saliva, urinary OT concentrations, especially detected from the first morning void, result from the net effect of accumulation and excretion of systemic OT over several hours. Morning urine should thus also be relevant for monitoring baseline OT levels as the summed excretion of elevated OT responses related to dyadic affiliative behavior [39] in callitrichid monkeys. In combination with behavioral data, this sampling method allows looking at the integration of social interactions with different partners at the hormonal level over time.

Methods

Subjects and housing

The study was performed using four common marmoset family groups (N = 3 to 5 individuals) and one male-female pair, amounting to a total of 19 adult individuals (9 females and 10 males, S1 Table in supporting information), aged between 2.3 to 9.5 years. No dependent offspring (nursing and being carried) were present at any time during the study. All groups were housed in standardized enclosures (depending on group size, one or multiple basic cage units; each measuring 2.4 m height x 1.5 m depth x 0.8 m width, with max. 3 animals per cage unit) at the Primate Station of the Anthropological Institute and Museum, University of Zurich. Each enclosure had a spacious outdoor area (measuring 2.4 m height x 2.7 m depth x 2.5 m width) and was equipped with a sleeping box, a water dispenser, several wooden climbing structures, an infrared lamp, and a mulch floor. The animals were housed under natural light with additional artificial light on a 12 h/12 h light–dark cycle and UV light (300W). Their diet consisted of a vitamin and calcium-enriched porridge in the morning, fresh fruits and vegetables over midday,

as well as gum and mealworms in the afternoon, and water was available ad libitum. A special cage unit for urine collection was attached to each home cage and only accessible in the morning during urine collection [64]. On urine sampling days, mealworms were fed in the urine cages in the morning instead of afternoon.

Urine sampling and OT analysis

For OT detection, morning urine samples of all 19 individuals were collected two to three times a week (alternating groups between 2 and 3 sampling days per week) over six consecutive weeks (total n = 275 samples). The sampling was restricted to morning void urine, in order to measure hormonal baseline values and to minimize confounding effects of circadian rhythms. This was done non-invasively either in a urine cage or with a plastic cup in the home cage, as described elsewhere [64]. Urine cages were made of transparent plastic panels and separated into six compartments with individual trap doors and access to a feeding trough at the backside. A wire-mesh and a metal panel with drains and adjustable vessels under each compartment allowed the collection of individual samples without contamination. Before the sampling period, animals were habituated over several weeks to enter and feed in the cage voluntarily. After awakening the group in the morning, animals were allowed to access the urine cage and kept in separate compartments until after urinating, but no longer than 15 minutes in total. The urine cages were rinsed carefully after each usage. Urine samples were labelled right after collection and stored immediately at -20°C in 1 ml portions until analysis.

OT was detected using ELISA technique in the Assay Services Unit of the Wisconsin National Primate Research Center in Madison, Wisconsin, according to the protocol by Seltzer and Ziegler [56] and Snowdon et al. [17]. Urine samples were run in duplicates at a volume of

200 µl. Prior to OT detection, samples were thawed, centrifuged, and extracted using solid phase extraction columns (Macherey-Nagel, 55–150 mm, WAT023501). Extraction columns were conditioned with 1 ml 100% methanol and 1 ml purified water. Samples were added (0.5 ml urine), washed with 1 ml 10% acetonitrile, 1% TFA (trifluoroacetic acid) in water, and eluted with 1 ml 80% acetonitrile in water. Samples were dried, reconstituted in 250 µl assay buffer, and added to the microtiter plates according to the directions provided with the assay kit (Assay Designs, Cat no. 901-153). The assay standard curve ranged from 6 to 1000 pg/ml and assay sensitivity was 6 pg/ml. Mean intra-assay coefficients of variation and inter-assay coefficients of variation were 5.85 % and 16.11 %, respectively. All OT concentrations were corrected for creatinine levels to control for variable urine concentration and log-transformed (log pg OT/mg Cr) to reach normal distribution prior to statistical analysis (Kolmogorov-Smirnov test for normality: $D(275) = 0.037$, $p = 0.33$).

Behavioral observations and analysis

In order to investigate the link between bond strength and OT synchrony, marmoset groups were observed for affiliative behavior along with the urine sampling over six weeks. We observed the groups 2-3 times per week on alternating days and recorded all social interactions based on continuous sampling in 10-minute focal observations for each individual within its group. Affiliative behaviors, including allogrooming (defined as using hand and/or mouth to pick through fur, face or mouth of another individual) and huddling (defined as sitting or lying in body contact with another individual), were used to estimate bond strength (the average over all dyadic affiliative events). Agonistic behavior was very rare and therefore not included in the analysis. All observations were performed by the same observer using an established protocol

(see also Koski and Burkart [65]; a test for inter-observer reliability with an independent observer resulted in 0.89 percent of agreement). An average of 173 ± 3.48 observation minutes were available for each individual. Urine samples were always collected the next morning, following observation days.

Statistical data analysis

Dyadic bond strength was estimated based on dyadic affiliation - mean rates of mutual grooming and huddling - for all 30 dyads, i.e. 5 breeder-breeder (mates), 18 breeder-helper (parent-adult offspring) and 7 helper-helper (siblings) dyads (S2 Table in supporting information). Durations of affiliative behaviors were corrected for observation time and combined into one log-transformed value (log[sec grooming + sec huddling]/sec observed). Dyadic OT synchrony was calculated as the Pearson correlation coefficient of the longitudinal OT fluctuations of two partners (N = 8 to 13 OT values per dyad), resulting in one synchrony value per dyad (S2 Table).

First, we analyzed variation of individual urinary OT levels between sex-status groups (female and male breeders and helpers) and variation of dyadic bond strength (mean dyadic affiliation) between breeder-breeder, breeder-helper and helper-helper dyads, using analysis of variance (R: one-way ANOVA; completely randomized design). Post-hoc tests between groups and effect sizes for ANOVA results were calculated using Scheffe-test and eta square, respectively.

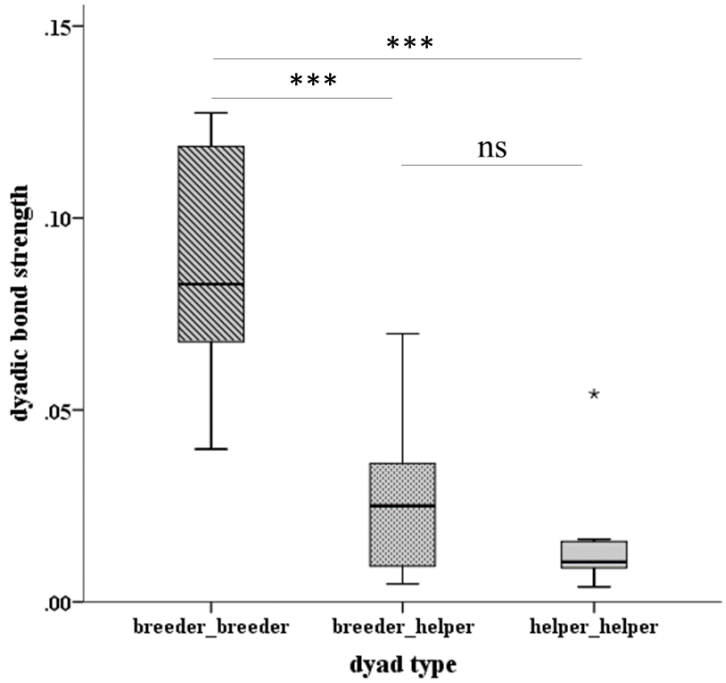
Second, we tested whether strongly bonded dyads showed higher OT synchrony and whether this relationship was affected by dyad type, using a permutation-based linear model (R version 3.0.3: lmPerm, Exact permutation analysis). The permutation step was included (i) to

compensate for small sample size, and (ii) to control for dependencies due to individuals participating in multiple dyads in a group, by randomly resampling dyadic OT synchrony and affiliation values across groups. Dyadic OT synchrony was the dependent variable and dyadic bond strength, dyad type, and the interaction dyadic bond strength*dyad type were included as factors.

Results

Mean individual baseline OT levels (mean ± SE) of female breeders, male breeders, female helpers, and male helpers were 2.99 ± 0.24, 2.85 ± 0.26, 2.53 ± 0.16, and 2.74 ± 0.25 (log pg OT/mg Crt), respectively. Differences between sex-status groups were non-significant (one-way ANOVA: $F(3, 15) = 0.84$, $P = 0.49$, $h^2 = 0.14$), but OT levels tended to be highest in female breeders.

Dyadic bond strength (rates of mutual dyadic affiliation) varied widely across all dyad types. Mean ± SE values for breeder-breeder, breeder-helper and helper-helper dyads were 0.087 ± 0.016, 0.026 ± 0.004 and 0.017 ± 0.006 (log sec/sec observed), respectively, and dyadic bond strength was significantly higher in breeder dyads than in the two other dyad types (one-way ANOVA and post-hoc Scheffe: $F(2, 27) = 18.43$; $P < 0.001$, $h^2 = 0.58$) (figure 1).



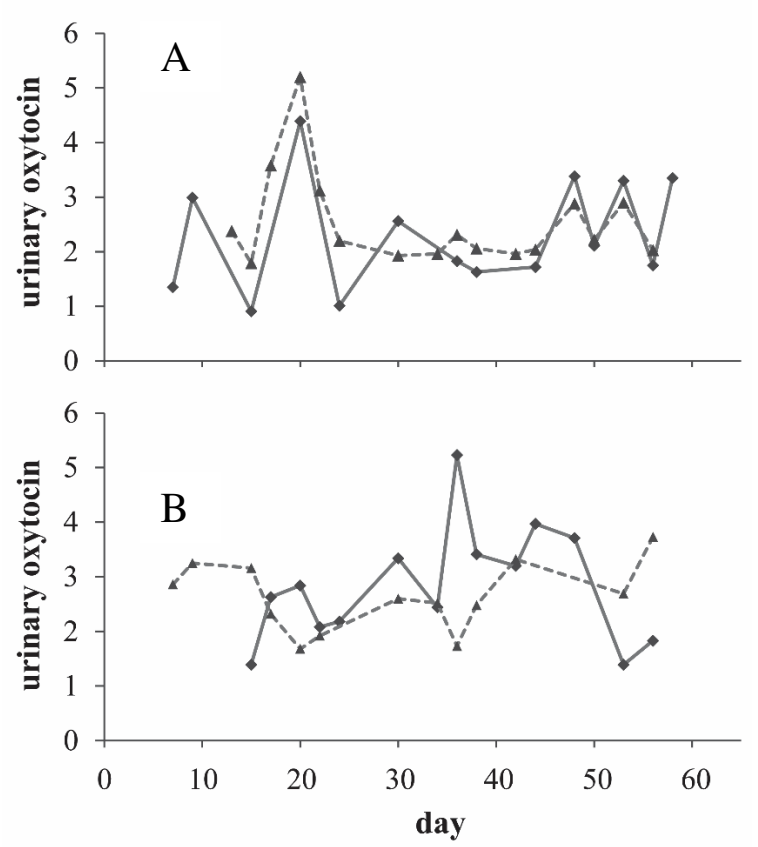
220

221 **Figure 1. Bond strength in different dyad types.** Variation in mean dyadic levels of affiliation
222 (dyadic bond strength) in 5 breeder-breeder, 18 breeder-helper, and 7 helper-helper dyads. Mean
223 dyadic affiliation is significantly higher in breeder-breeder dyads compared to the other dyad
224 types. (single column fitting image)

225

226 Dyadic OT synchrony values, calculated as Pearson correlation coefficient for each dyad,
227 varied between r = -0.55 and 0.82 with 5 significant correlations (16.6 % of the total 30 dyads).
228 The permutation ANOVA on dyadic OT synchrony revealed an effect of dyadic bond strength
229 (mean dyadic affiliation), and dyad type, whereas the interaction dyadic bond strength*dyad type
230 had no effect (Table 1). Figure 2 illustrates longitudinal OT levels and OT synchrony of two
231 strongly (2A) and two weakly (2B) bonded individuals. The link between dyadic bond strength
232 and OT synchrony was significant across all dyad types (Table 1, Linear model estimate = 10.14,

233 P < 0.01). It was equally strong in breeder-breeder and helper-helper dyads, but weaker in mixed
234 breeder-helper dyads, where variation was broader (Figure 3).



235

236 **Figure 2. Temporal correlation of urinary OT in two dyads.** OT levels (log pg OT/mg Crt) in
237 a strongly bonded (A) and a weakly bonded (B) dyad; both dyads are composed of a female
238 breeder (solid lines) and a male helper (dashed lines). Dyadic OT synchrony values for A and B
239 are 0.79 and -0.41, respectively. (1.5-column fitting image)

240

241 Figure 3 shows the relationship between dyadic OT synchrony and dyadic bond strength.
242 The right shift of the regression line in breeder-breeder dyads (solid red regression line)
243 compared to other dyads reflects the relatively higher levels of dyadic affiliation in breeders.
244 However, this shift did not affect the significant link between OT and bond strength across dyad

types, as indicated by the non-significant interaction between dyadic bond strength and dyad type (Table 1). The residual distribution of the linear model was normal (Shapiro-Wilk normality test: W = 0.97, P = 0.46), suggesting a good model fit to the data.

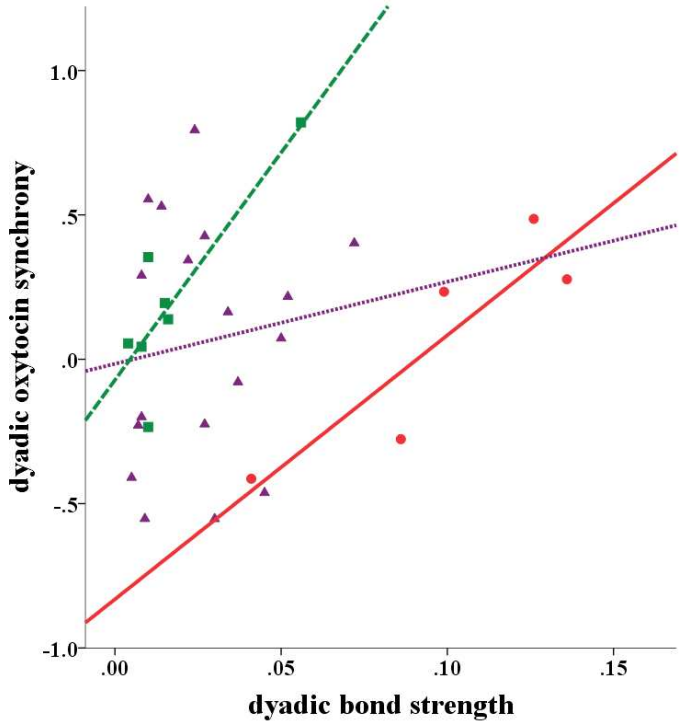


Figure 3. Positive link between dyadic bond strength and OT synchrony. The pattern shows a similarly strong trend for breeder-breeder (dots, solid red line; R^2 Linear = 0.582) as well as helper-helper dyads (squares, dashed green line; R^2 Linear = 0.713), but a weaker trend for mixed breeder-helper dyads (triangles, dotted purple line; R^2 Linear = 0.170). (single column fitting image)

Discussion

In this study, we explored whether dyadic bond strength in group-living marmoset monkeys was linked to synchronized fluctuations in longitudinal OT levels. We found that strongly bonded dyads exhibit synchronized fluctuations of urinary baseline OT over time, both

in breeder-breeder dyads and other dyad types. These findings are consistent with the dyad-specificity of OT responses to affiliative behavior in chimpanzees [18], because they suggest that an individual's OT profile is particularly determined by its affiliative interactions with strongly bonded group members, whereas interactions with other group members influence its OT profile less. Over time, this arguably leads to the reported temporal correlations and synchronization of OT profiles in bonded dyads, reflecting how much the individuals engaged in affiliative behavior with each other. Furthermore, the findings suggest the existence of differentiated dyadic relationships in marmosets, which is consistent with the species' characteristic stable and long-lasting relationships between mate pairs [26]. However, strongly bonded dyads with synchronized OT fluctuations were not exclusively found among breeding pairs, as the pattern also extended to breeder-helper and helper-helper dyads.

The relationships between callitrichid group members other than the breeding pair are mainly shaped by their cooperatively breeding lifestyle [66], but the function of differentiated relationships as described here remains to be established. Stronger relationships between same-sex helpers may provide benefits during dispersal [67], whereas close breeder-helper relationships may result in increased future allomaternal care by helpers (in particular if breeders are more responsible for establishing and maintaining these relationships) or in longer periods of acceptance in the natal group (in particular if helpers are more responsible for establishing and maintaining these relationships). Alternatively, differentiated relationships between group members other than breeding pairs might simply reflect the extent to which an older individual was engaged in caring for a younger individual during infancy [68], or finally occur as a by-product of the mechanism that has evolved in this species to maintain stable and long lasting socially monogamous relationships between mates. Aggressive interaction were too rare to

include them in our evaluation of dyadic relationships. However, earlier studies have shown that breeding competition among females, leading to severe aggression and occasional infanticide, is part of callitrichid group life, especially in the wild [69-72], and may thus also play a role in shaping dyadic relationships.

The link between dyadic OT synchrony and bond strength (affiliation) was found to be positive across all dyad types but it was strongest in breeder-breeder and helper-helper dyads, and, in breeder dyads, the correlation was relatively shifted to the right (Figure 3). This pattern shift originated from the significantly higher levels of dyadic affiliation in breeders compared to other dyads, which are probably partly resulting from breeding pairs spending more time in proximity during periods of increased sexual activity during the female ovarian cycle [73]. Whether our dyadic proximity estimates for breeding pairs are comparable to those in more heterogeneous groups or in the wild, where the turn-over for breeding positions is also faster [74], remains unknown. Compared to breeder-breeder and helper-helper dyads, the link between OT synchrony and affiliation was weaker in mixed breeder-helper dyads, possibly because breeder-helper relationships are more heterogeneous [71]. Importantly, we can rule out high relatedness as a factor driving OT levels or OT synchrony. All members of a group, except the breeding pairs, were directly related and our pattern does not indicate better synchronization between siblings (including twins) or parent-offspring dyads than between mates (Figure 3).

A potential error source of variation in the observed link between synchronized longitudinal OT fluctuations and dyadic affiliation is sexual cycling in reproductively active females, since estrogen is known to have a stimulatory effect on OT [48, 49]. This mainly pertains to breeding females but may also include subordinate female helpers. Sexual cycling in subordinate females is usually inhibited [75, 76] but reproductive suppression occasionally fails

in daughters after they reach puberty [35, 76, 77]. However, removing all dyads that contain breeding females from the data set does not erase the significant effect of dyadic bond strength in the linear model analysis (Linear model estimate=10.99, p=0.02).

The pattern of dyadic synchronization as presented in our data is consistent with studies on marmoset dyadic behavioral synchrony [78] and on human parent-infant bonding: Human infants, whose fathers had been previously treated with exogenous OT, showed an indirect increase of peripheral OT levels after interaction with the father [5, 79] and longitudinal synchronization between individuals occurred on the behavioral level [5, 80, 81]. Finally, in humans, menstrual hormonal synchrony has been described among close friends and in families where mutual contact and exposure were high [82, 83]. These analogous patterns suggest that temporal coordination between individuals can occur on the behavioral as well as on the physiological level and might hence represent a more general functional mechanism in social bonding.

Conclusion and implications

Our results show that, in common marmosets who live in family groups, more strongly bonded dyads have more synchronized longitudinal fluctuations of urinary baseline OT levels compared to less strongly bonded dyads. This patterning indicates that, despite the high cohesiveness among marmoset groups, OT responses are to some extent dyad-specific. Our results are thus consistent with the positive link between dyadic affiliative behavior and elevated urinary OT in strongly bonded chimpanzees, and with a possible role of OT as a mediator of social interactions and bonding, as suggested by Crockford et al. [18] and Wittig et al. [19]. Between-individual OT effects may thereby be established and maintained via positive feedback

mechanisms triggered by affiliative [18], but also gaze [60, 84] or voice [7] interactions. Intriguingly, highly affiliative dyads with synchronized OT fluctuations were not exclusively found among breeding pairs, but also among breeder-helper and helper-helper dyads. The presence of differentiated relationships among non-breeding dyads raises the question about the ultimate function of such relationships.

Finally, hormonal synchronization between two individuals may represent a hitherto neglected dimension in the establishment and maintenance of social relationships. It remains to be established in future studies whether such shared longitudinal fluctuations of OT also lead close individuals to share mood shifts and whether such a connection could provide a hormonal link to psychological and emotional consequences of bonding.

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525

Supporting Information Legends

S1 Table. Individual information. Individual group membership, name, sex, status, and age of all 19 individuals that were part of this study.

S2 Table. Dyadic information. Dyad-specific group membership, composition, OT synchrony, and affiliation values of all 30 dyads that were part of this study.

Tables

Table 1. Permutation-based linear model results table. Analysis of the influence of dyadic bond strength (mean dyadic affiliation) and dyad type on dyadic OT synchrony in group-living marmoset dyads. . Dyad type levels are coded as BB (breeder-breeder), BH (breeder-helper), and HH (helper-helper). Parameter estimates: factor levels with 0 are compared with remaining factor levels. Bold: $p < 0.05$.

model factor	F	df	p-value	factor level	estimate	p
Dyadic bond strength	**9.84**	**1**	**0.004**		**10.14**	**0.004**
Dyad type	**2.90**	**2**	**0.075**	**BH**	**-0.39**	**0.024**
				HH	0.03	0.361
				BB	0	0
Dyadic bond strength*dyad type	0.89	2	0.424	BH	-2.90	0.322
				HH	-4.24	0.286
				BB	0	0